BARD Final Report IS-4541-12

Novel regulation of transpiration by sugar signals within guard cells

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Scientific abstract of the project

Water is the major limiting factor in agriculture and stomata, composed of two guard cells and the pore they circumscribe, are the chief gates controlling plants' water loss. The prevailing century old paradigm was that sugars act as an osmoticum in guard cells, contributing to the opening of the stomata. In contrast, we discovered that sugars close stomata and the closure is mediated by the sugar-sensing enzyme hexokinase (HXK) that triggers the abscisic acid (ABA)-signaling pathway within the guard cells. This new discovery suggests a sugar-sensing mechanism within guard cells that controls stomatal closure, and supports the existence of a stomatal feedback mechanism that coordinates photosynthesis with transpiration.

Summary Sheet

PubType	IS only	Joint	US only
Book Chapter	0	0	1
Patent	1	0	0
Review Article	3	0	0
Reviewed	3	1	1

Contribution of the collaboration between the laboratories to the research

The granot lab is working on sugar sensing in plants for about two decades and its new discovery that sugars close stomata, in contrast to the century-old paradigm that sugars open stomata, took the lab into a new field that was poorly known to the Granot lab at that time. The laboratory of Sally Assmann was, and is among the leading labs in the field of stomata function. Fortunately Sally was willing to join forces and pursue our new discovery. She contributed tremendously to the development of the research proposal and used our lines to further explore into the molecular mechanisms. Seeds of the transgenic Arabidopsis and tomato plants created in Granot's lab were provided to Assmann's lab and were used for studies on the effect of sugars and HXK on Arabidopsis stomatal behavior and ion channels. The labs exchanged results and communicate frequently to discuss the results and the Israeli PI visited and spent several days in the lab of Sally Assmann at Penn State University on May 2015 to discuss the project, publications and future plans. We have one joint publication and at least one more joint publication will arise from this collaboration.

Major Achievements

When we started this research the common knowledge was that sugars are osmolytes that open stomata. Our discovery that sucrose closes stomata and that the closure is mediated by HXK faced strong opposition by the conservative hypothesis on the stomatal opening role of sugars. Therefore, a primary goal was to accumulate sufficient amount of data in order to convince the scientific community that our hypothesis on the closing role of sugars and HXK in guard cells is correct. This goal was met and a manuscript describing our discoverywas published in *The Plant Journal* (Kelly et al., 2013), and was recommended by F1000Prime as being of special significance in its field (http://f1000.com/prime/718017597?bd=1&ui=113252)

The article shows also that the regulation of stomatal closure by sugars and HXK is mediated by ABA, and involves nitric oxide production (NO) within guard cells. This first publication was followed by several reviews (listed below in the list of publications emanating from this study) and we believe that it is now widely accepted that sugars can close stomata, thus coordinating photosynthesis with transpiration rates.
Unlike expression of HXK in guard cells, expression of HXK in mesophyll cells had no effect on stomatal movement further confirming our discovery.

To study the role of sucrose and *AtHKX1* in guard cell K^+ channel regulation and stomatal movement in Arabidopsis, the Assmann lab performed stomatal movement and patch clamp experiments in wild-type and *AtHXK* overexpression lines (constructed by the Granot lab) under different growth conditions. Based on the results from tomato, it was hypothesized that sucrose and/or its invertased product glucose could inhibit stomatal opening in both wild-type and *AtHXK1* overexpression lines. It was also hypothesized that the *AtHXK1* overexpression lines should have smaller aperture sizes in the absence and the presence of external surcose, and have an enhanced response to sucrose treatment as compared to the wild-type. Because inhibition of guard cell inward K^+ channels can inhibit stomatal opening, it was further hypothesized that sucrose or glucose could inhibit these channels.

Experiments were performed first on Arabidopsis plants grown in the growth chamber under high humidity conditions. We found that the constitutive *(35SHXK)* and guard cell specific *AtHXK1* overexpression lines (*GCHXK*) showed consistently smaller aperture sizes compared to the wild-type, and smaller inward K^+ currents in the patch clamp study. However, we did not see any effect of surcose on stomatal movements or K^+ channel activity in either the wild-type or the overexpression lines.

To test if the growth condition could affect sucrose responsiveness, we repeated the stomatal opening and closure experiments using WT and *35SHXK* overexpression lines grown under greenhouse conditions. Interestingly, we found that sucrose and glucose could inhibit stomatal opening and promote stomatal closure in these plants, which is consistent with the observation in tomato.

There are many differences between greenhouse and growth chamber conditions, including day length, light intensity, humidity, temperature, and soil type. We began by manipulating the relative humidity, which is thought to affect plant ABA levels. Plants were grown in growth chambers under short day conditions and under high and low humidity conditions. Consistent with our hypothesis, we found that WT and *35SHXK* plants both responded to sucrose inhibition of stomatal opening when grown under low humidty conditions, but not when grown under high humidity conditions. We also tested the effect of light quality on stomatal opening using low humidity grown plants, and found that the sucrose inhibitory effect was observed under the condition 25 μmol s^{-1} m^2 blue light plus 125 μmol s^{-1} m^2 red light, but not under 125 μmol s^{-1} m^2 red light, suggesting a connection to the specific, phototropin-mediated blue light response of guard cells. Future plans include patch clamp experiments to determine sucrose effects on K^+ currents in plants grown under low humidity conditions.

We further explored the role of HXK in the response of stomata to humidity. It is well known that low humidity stimulates stomatal closure, but, despite many efforts, the mechanism that mediates this effect is not known. Once we established that sugars stimulate stomatal closure via guard cells HXK, we anticipated that this discovery may provide a logical biological explanation for the effect of low humidity on stomata, as follows. The immediate response of low humidity is increased transpiration. Increased transpiration is bound to carry more sugars toward the guard cells. These sugars may be sensed by HXK and stimulate stomatal closure.

To test this hypothesis we used Arabidopsis WT and the well characterized guard cell specific *AtHXK1* overexpressing lines (GCHXK – Kelly *et al.,* 2013) and followed the stomatal response when the plants were transferred from high humidity (>95%) to low humidity (50%). The response of the stomata was followed by continuous thermal imaging. As expected, the transfer of plants to low humidity was accompanied by immediate drop in leaf temperature due to the increased transpiration (that cools the leaves), but within less than 10 minutes, the drop in leaf temperature ceased and the temperature started to increase as a result of stomatal closure. As predicted, the temperature drop of the leaves of GCHXK plants ceased faster (within 5 min) and remained higher than that of the control plants. Furthermore, adding glucose, the substrate of HXK, one hr prior to the thermal imaging enhanced the effect. We therefore believe that sugars and HXK mediate the response of guard cell to changes in humidity.

The role of sugar and HXK in the response (closure) of stomata at low humidity was further tested with citrus plants (*Citrus sinensis* 'Washington' sweet orange × *Poncirus trifoliata*), expressing HXK specifically in guard cells. We have shown that HXK reduces stomatal aperture and transpiration at low humidity (high VPD - vapor pressure difference) thus supporting our hypothesis that the effect of low humidity is mediated by sugar and HXK within guard cells. These results were published in Lugassi et al., 2015.

The effect of HXK expressed in guard cells on water use efficiency (WUE) was examined using lysimetry and gas exchange (Licor). Both tomato and Arabidopsis plants expressing *AtHXK1* in guard cells exhibited significant increase in WUE. We also tested the effect of a lower amount of irrigation on yield. Tomato plants were grown in a semi-commercial set up and were irrigated at 100%, 75% and 50% of the regualr irrigation level. Tomato plants with guard cell specific *AtHXK1* overexpressing lines had higher fruit weight compare to the WT plants.

Increased WUE was also observed in the citrus plants expressing HXK in guard cells (Lugassi et al., 2015), supporting our hypothesis that increased expression of HXK in guard cells improves the coordination between photosynthesis and transpiration.

Publications for Project IS-4541-12

Status	Type	Authors	Title	Journal	Volume: Pages	Year	Country
Published	Review Article	*Granot, D., David-Schwartz, R. and Kelly, G.*	Hexose kinases and their role in sugar-sensing and plant development	***Front Plant Sci***	4 : 44	***2013***	IS only
Published	Reviewed	*Kelly, G., Moshelion, M., David-Schwartz, R., Halperin, O., Wallach, R., Attia, Z., Belausov, E. and Granot, D.*	Hexokinase mediates stomatal closure	***The Plant Journal***	75 : 977-988	***2013***	IS only
Published	Reviewed	*Kelly, G., Sade, N., Attia, Z., Secchi, F., Zwieniecki, M., Holbrook, N.M., Levi, A., Alchanatis, V., Moshelion, M. and Granot, D.*	Relationship between Hexokinase and the Aquaporin PIP1 in the Regulation of Photosynthesis and Plant Growth	***. PLoS One***	9 : e87888	***2014***	IS only
Published	Review Article	*Lawson, T., Simkin, A.J., Kelly, G. and Granot, D.*	Mesophyll photosynthesis and guard cell metabolism impacts on stomatal behaviour	***New Phytol***	203 : 1064-1081	***2014***	IS only
Published	Reviewed	*Yu, Y. and Assmann, S.M.*	Metabolite transporter regulation of ABA function and guard cell response	***Mol Plant***	7 : 1505-1507	***2014***	US only
Published	Reviewed	*Misra, B.B., Acharya, B.R., Granot, D., Assmann, S.M., and Chen, S.*	The guard cell metabolome: functions in stomatal movement and global food security	***Front Plant Sci***	6 : 334	***2015***	Joint
Published	Book Chapter	*Zhu, M., Jeon, B., Geng, S., Yu, Y., Balmant, K., Chen, S., and Assmann, S.*	Preparation of Epidermal Peels and Guard Cell Protoplasts for Cellular, Electrophysiological, and -Omics Assays of Guard Cell Function	***Plant Signal Transduction***	: 89-121	***2016***	US only
Published	Patent	*Granot D. Gilor K. and Moshelion M.*	Methods of modulating stomata conductance and plant expression constructs for executing same		:		IS only
Published	Review Article	*Granot, D., Kelly, G., Stein, O. and David-Schwartz, R.*	Substantial roles of hexokinase and fructokinase in the effects of sugars on plant physiology and development	***J Exp Bot***	65 : 809-819	***2014***	IS only
Published	Reviewed	*Lugassi, N., Kelly, G., Fidel, L., Yaniv, Y., Attia, Z., Levi, A., Alchanatis, V., Moshelion, M., Raveh, E., Carmi, N., and Granot, D.*	Expression of Arabidopsis hexokinase in citrus guard cells controls stomatal aperture and reduces transpiration	***Frontiers in Plant Science***	6 :	***2015***	IS only